A role for the Hippo pathway in a non-canonical apoptotic program

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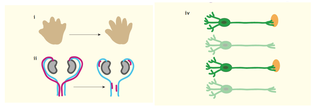
Abstract

We have previously described a non-canonical apoptotic program in the nematode *C. elegans*, Compartmentalized Cell Elimination (CCE), through which two complex embryonic cells, an epithelial cell (the tail-spike cell) and a set of sensory neurons, die in a tripartite fashion. From a candidate gene screen, we found that mutants for *egl-44*, which encodes a transcription enhancer factor of the TEA domain (TEAD) class, have CCE defects. TEADs are key transcription factors of the Hippo pathway, an evolutionarily conserved signaling network that serves in cell proliferation and differentiation, organ growth, embryogenesis, and wound healing. Dysregulation of the Hippo pathway is linked to cancer, and many other diseases. In mammals, the YAP (Yes-associated protein (YAP))/TAZ, also part of the Hippo pathway, are transcriptional coactivators that bind to TEAD 1–4 transcription factors. We found that mutants for *C. elegans yap-1* also have CCE defects. Our preliminary genetic data link a highly important signaling pathway to a novel form of cell death. Our future studies include determining the transcriptional target of the EGL-44/TEAD/ YAP-1 module.

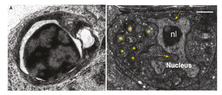
Background Concepts

PCD is an important developmental event



Programmed cell death (PCD) is a form of pre-destined, genetically programmed, evolutionarily conserved cell elimination that has many functions. It plays roles in development, eliminating unwanted cells, and stress response.

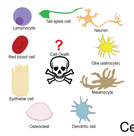
There are different types of PCD



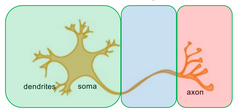
There are multiple forms of PCD. The best described is apoptosis, which is characterized by cellular shrinking, cellular rounding and chromatin condensation. Genetically apoptosis requires caspase proteases with the main regulator being human caspase-3.

Another type of PCD is Linker-type cell death (LCD) first described in the nematode *C. elegans*. LCD is a caspase-independent, non-apoptotic form of cell death.

Elimination of morphologically complex cells is poorly understood



Cellular diversity



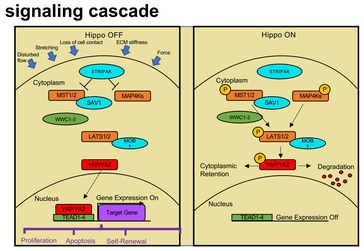
Compartment specificity

Cellular diversity is a fundamental feature of the metazoan body. Different cells perform different functions and have a range of morphologies. Little is known how such diversity influences how a cell dies.

Morphologically complex, or polarized cells are characterized by distinct compartments, such as the cell body, axon and dendrites of neurons. These compartments differ in their subcellular architecture and surrounding microenvironment.

The Hippo signaling cascade

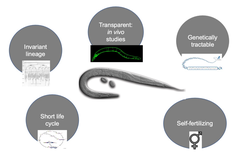
The Hippo signaling pathway is an evolutionarily conserved signaling network and a major regulator of cell proliferation, apoptosis, movement and fate. Dysregulation can cause a variety of diseases, including cancer. Recent work implicates this cascade in neurodegeneration.



TEADs (Transcriptional enhanced associate domain transcription factors) integrate with and coordinate various signal transduction pathways including Wnt, TGFβ, and EGFR and Hippo pathways. TEADs are the key transcription factors of the Hippo pathway. How TEAD transcriptional activity is modulated, such as by post-translational modifications or nucleocytoplasmic shuttling, and whether this is Hippo-dependent or Hippo-independent is an area of increasing interest.

YAP (Yes-associated protein) and its paralog TAZ are the key effectors of the Hippo signaling cascade. Their regulation by the Hippo kinase cascade and the back-and-forth translocation of YAP between the nucleus and the cytoplasm serve as a central mechanism for sensing mechanical forces and regulating mechanotransduction, When the Hippo pathway is off, YAP translocates to the nucleus where it can drive co-transcriptional activity. In addition to other roles, YAP can both inhibit or induce different forms of cell elimination, including apoptosis, autophagy, ferroptosis and pyroptosis.

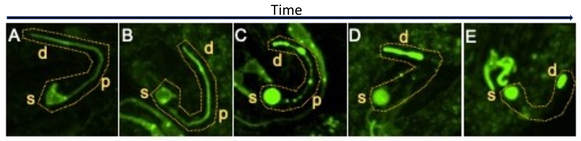
Study System



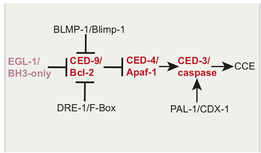
The nematode C. elegans is a powerful genetic model organism for several reasons: invariant lineage, transparent, genetically tractable, short life cycle, self-fertilizing. Here, the genetic program for apoptotic cell death was first described.

Background Results

Compartmentalized Cell Elimination (CCE) is a novel developmental program of cell death



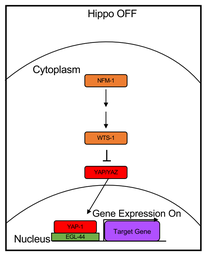
CCE observed in tail-spike cell: intact, severing of soma-process junction, beading of proximal process, distal process retraction, prior to phagocytosis.



CCE is a form of embryonic programmed cell death that shows hallmarks of developmental pruning. CCE also occurs in a set of sensory neurons in the worm and is this potentially universal and maybe a broad phenomenon.

Genetically, CCE is dependent on the main *C. elegans* caspase CED-3, but independent of one of its upstream regulators, EGL-1/BH3-only, making it a non-canonical form of apoptosis. CCE is also therefore a novel setting to discover new regulators of PCD

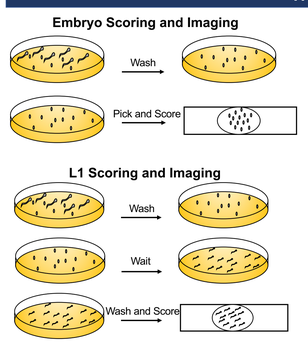
Central Question

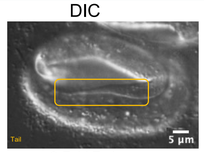


Is the Hippo signaling pathway involved in the novel cell death program of Compartmentalized Cell Elimination (CCE)?

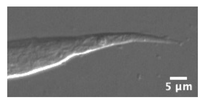
The Hippo signaling pathway is conserved and has been shown in *C. elegans* to be involved in the maintenance of cell polarity (Lee et al. 2019) as well as thermotolerance, aging (Iwasa et al. 2013), neuronal cell fate (Wu 2001), and host defense (MA et al. 2020). However, there are no reports of this pathway being involved in cell death in *C. elegans.*

Methods





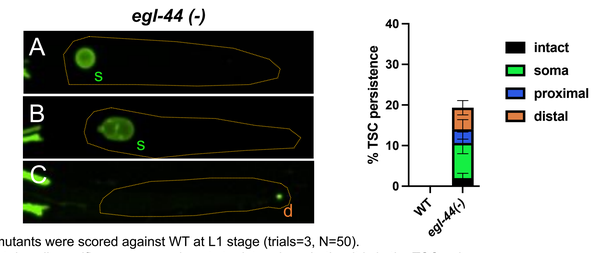
3-Fold Embryo CCE



L1 Tail Tip
Cell should be gone

Recent Results

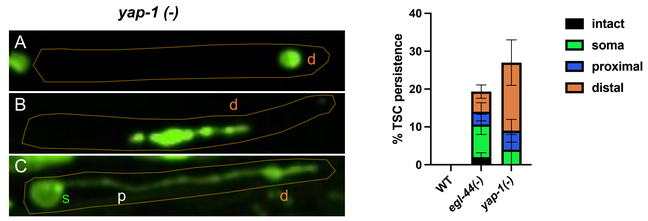
egl-44/TEAD mutants have mainly soma elimination defects



egl-44 (-) mutants were scored against WT at L1 stage (trials=3, N=50).
We will next do cell specific rescue experiments to determine whether it is in the TSC or in a surrounding cell. Do *yap-1 (-)* mutants phenocopy *egl-44 (-)* mutants?

Recent Results continued

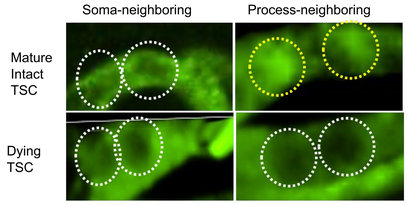
yap-1/YAP mutants have mainly TSC process elimination defects



yap-1 (-) mutants were scored against WT at L1 stage (trials 3, N=50).
We will next do cell specific rescue experiments to determine whether it is in the TSC or in a surrounding cell.

yap-1 is not expressed in the intact or dying TSC

YAP-1 expression and localization were examined in the 3-fold embryo using a translational reporter using the endogenous reporter as well as a TSC membrane marker in a WT background.



YAP-1 is nuclear in the cell neighboring the intact TSC process

Outstanding questions/Future directions

What is EGL-44/TEAD's role in CCE?

Does EGL-44 function cell autonomously?
- Cell-specific rescue, Expression

Is the persisting soma in egl-44 mutants living?
- Double mutant with engulfment mutant (*ced-5*)

What gene(s) does EGL-44 target?
- ChIP Seq

What is YAP-1/YAP's role in CCE?

Does YAP-1 function cell autonomously?
- Cell-specific rescue

Does YAP-1 repress killing?
- Nuclear localization at earlier stages

Does YAP-1 promote killing?
- Nuclear localization at death initiation

Are the persisting process fragments phagocytosed?
- Phagocyte marker

Do Hippo pathway members regulate YAP-1 role in CCE?
- Test *wts-1*/LATs and *nfm-1*/Merlin overexpression

What is YAP-1's corresponding transcription factor?

What gene(s) does YAP-1 help regulate?

Are EGL-44/TEAD and YAP-1/YAP associated in CCE regulation?

egl-44; yap-1 double mutants
Other *egl-44* and *yap-1* alleles

References

- Ghose P, et.al,. EFF-1 fusogen promotes phagosome sealing during cell process clearance in Caenorhabditis elegans. Nat Cell Biol. 2018 Apr;20
- Juanez K, Ghose P. Repurposing the Killing Machine: Non-canonical Roles of the Cell Death Apparatus in *Caenorhabditis elegans* Neurons. Front Cell Dev Biol. 2022 Feb 14

Acknowledgements

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